BLACKBOX® LASV IgG ELISA Kit (Lassa Virus, IgG)

For Research Use Only (RUO)

REF: ELG.004

Lot No. (See product label)

Size: 96-wells

Principle: IgG ICB (Immune Complex Binding) ELISA

Type: Qualitative

Storage: Conjugate and HRP-Streptavidin have to be stored at -20°C. Store all other kit components at 2°C - 8°C.

Intended Use:

The BLACKBOX® LASV IgG ELISA Kit is intended for qualitative detection of IgG antibodies to LASV in human serum.

In conjunction with the BLACKBOX® LASV IgM ELISA Kit, the assay provides serological evidence of an acute or past infection with LASV. Test results have to be critically assessed with reference to clinical symptoms, available anamnestic information and the results of other diagnostic tests performed.

The kit is not intended for self-testing. Assay performance characteristics have not been established for automated instruments.

General Description: Lassa Fever

Lassa fever is an infectious disease endemic in several West African countries including Guinea, Liberia, Nigeria, and Sierra Leone [1]. The causative agent for this zoonotic disease is a virus of the *Arenaviridae* family, the Lassa Virus (LASV). Virus transmission to humans occurs either by inhalation or ingestion of excrements of the natural reservoir host, the multimammate rat *Mastomys natalensis*, or by direct contact with body fluids from infected patients. In about 20% of infected individuals, symptoms like fever, weakness, headache, sore throat, cough, nausea, vomiting, diarrhoea and muscle, abdominal and chest pain develop after an incubation period of 6 – 21 days; approximately 80% of infections remain asymptomatic [1]. Due to the severity of the disease, the limited therapeutic options and the high risk of human to human transmission, LASV is classified as a virus of the highest biological risk class (Biosafety Level 4).
LASV-specific IgM antibodies usually become detectable towards the end of the first week post onset of symptoms and may persist for months to years [1]. LASV-specific IgG antibodies emerge shortly after or concurrently with LASV-specific IgM antibodies [2] and may persist for decades [1].

Test Principle

The BLACKBOX® LASV IgG ELISA Kit is based on the patented IgG Immune Complex Binding (ICB) ELISA technology [2, 3]. Diluted rabbit Control sera and patient serum samples are co-incubated together with a biotinylated recombinant LASV antigen in a microwell plate coated with a recombinant IgG immune complex specific capture molecule. During the incubation time, immune complexes are formed which bind specifically and with high affinity to the capture molecule. All antibodies not binding to the antigen, as well as any excess labeled antigen, are removed in the subsequent washing step. The bound IgG/antigen immune complexes are visualized by subsequent application of horseradish peroxidase (HRP)-labeled streptavidin and the colorimetric HRP substrate TMB. After stopping the enzymatic reaction, the assay result is generated by measuring the optical density of the solution in the well at 450/620 nm.

Reagents and materials provided in the kit

Component	Supplied amount/ packaging	Color coding	Storage
Microwell plate (IgG)	12 strips in sealed aluminium pouch with desiccant bag	n.a.	2°C - 8°C
Positive Control, ready-to-use	350 µl in a 0.5 ml vial	red cap	2°C - 8°C
Negative Control, ready-to-use	700 µl in a 0.5 ml vial	white cap	2°C - 8°C
Sample Dilution Buffer (SDB)	100 ml in a 125 ml bottle	clear cap	2°C - 8°C
Conjugate Dilution Buffer (CDB)	28 ml in a 30 ml bottle	blue cap	2°C - 8°C
10X Wash Buffer	100 ml in a 125 ml bottle	clear cap	2°C - 8°C
Conjugate (biotinylated recombinant LASV antigen)	30 µl in a 0.5 ml vial	blue cap	-20°C
HRP-Streptavidin	25 µl in a 0.5 ml vial	clear cap	-20°C
Substrate	14 ml in a 15 ml amber bottle	amber cap	2°C - 8°C
Stop Solution	14 ml in a 15 ml bottle	clear cap	2°C - 8°C
Adhesive foil	2 pieces	n.a.	n.a.
Instruction for use	1	n.a.	n.a.

Table 1. Reagents and materials provided in the kit.

The kit allows the performance of 96 reactions, including Positive and Negative Controls. For analysis of small numbers of patient samples, provided reagents are sufficient for 12 independent tests (1 strip per test, 5 patient samples per strip).

For information on reagents' shelf life and handling/security instructions see page 8 of this manual.

Materials/instruments required but not supplied in the kit

For preparation of 1X Wash Buffer:
Deionized water
Graduated cylinder
Pipetboy
Glass or plastic pipettes for volumes up to 25 ml

For preparation of other reagents/serum samples and assay performance:
Pipettes for volumes up to 10 µl, 100/200 µl and 1000 µl
Pipette tips for volumes up to 10 µl (short, graduated tips are recommended), 100/200 µl and 1000 µl
Microcentrifuge tubes
Paper towels/absorbent paper
Timer
ELISA plate reader (450 nm, 620 nm)

Optional:
Eight-channel pipette
Reagent reservoirs
Dispenser pipette and tips
Microplates with V bottom
Automated ELISA plate washer
Incubator set to 23°C

Specimen collection, preparation, storage and handling

The BLACKBOX® LASV IgG ELISA Kit has been developed using human sera. Assay performance was not tested using whole blood, plasma or other specimens. Use of hyperlipemic, hemolyzed, icteric or contaminated sera may cause erroneous results.

For serum preparation, blood samples must be collected by approved venipuncture procedures, and carried out by qualified personnel using appropriate collection tubes, designed to allow blood clotting. For clotting, incubate the blood sample for 30 min at RT (alternatively: overnight at 4°C). After clotting, centrifuge (1400 x g, 10 min, 4°C), then aseptically transfer the supernatant (serum) to a fresh sterile tube.

Serum samples can be kept at RT for short periods of time (< 8 hours). For storage, serum samples should be refrigerated (4°C, < 6 months) or frozen (-20°C or -80°C, long term storage). Repeated freeze/thaw cycles should be avoided. It is recommended to ship serum samples on dry ice. After thawing, serum samples must be mixed gently but thoroughly.

If inactivation is necessary, please apply the following protocol:
Prepare a 2% Triton X-100/PBS solution (not supplied) and mix it 1:1 with the sera. Incubate at 37°C for 1 hour. Please note, as this dilutes the sera in half, twice the volume of these inactivated sera will need to be used when preparing the sample dilutions for the assay to achieve the correct dilution factor, e.g. mix 20 µl inactivated serum + 480 µl SDB.

Test Procedure

General remarks

- Perform all pipetting steps at room temperature (20°C – 25°C) using calibrated, well maintained pipettes and strictly follow the ELISA procedure protocol described below. Deviations in assay parameters like volumes, incubation times and incubation temperatures may cause invalid results.
- Mix all reagents gently but thoroughly before use.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Upon arrival, **store the Conjugate and the HRP-Streptavidin at -20°C**. For Conjugate dilution (see below) remove the necessary amount of Conjugate from the vial and immediately place the residual Conjugate back in the freezer. As the Conjugate storage buffer contains glycerol, it is not necessary to thaw the Conjugate solution before pipetting. For the **HRP-Streptavidin, freeze-thaw cycles should be minimized**, therefore aliquotation into small volumes (e.g. 5 µL), at first thaw, is recommended if multiple assays will be carried out with a single kit.
- **CDB and SDB** should be chilled, because the formation of immune complexes takes place **at 4°C.**
- To prevent condensation, the **Microwell Plate IgG** (sealed in an aluminium pouch with a desiccant bag) must be **equilibrated to room temperature (20°C – 25°C) at least 30 minutes** before opening the package to remove the required number of microwell strips. Unused microwell strips can be stored in the presence of the desiccant bag at 4°C in the re-sealed aluminium pouch.
- **Wash Buffer, Substrate and Stop Solution** should be equilibrated to room temperature **(20°C – 25°C)** before use.
- Plate washing can be performed manually using a multi-channel pipette, however it is preferable that an automated plate washer is used. In both cases, quantitative removal of wash solution after the washing steps is mandatory. Remaining buffer can be removed by tapping the microplate face-down on an absorbent paper towel. **Important: When using an automated plate washer, account for the additional volume needed for system priming when calculating the required volume of 1X Wash Buffer.**
- Avoid cross-contamination of wells during all pipetting and washing steps.
- Avoid the formation of air bubbles during all pipetting steps. Particularly, air bubbles present during the OD measurement may cause false readings. If air bubbles do occur during the development step, these can be burst before OD measurement by carefully touching them with a dry, fresh pipette tip.

Preparation of reagents and specimen

- **1X Wash Buffer.** Wash Buffer is provided in the kit as a 10X stock solution. Prior to use, inspect this solution for salt crystals in the bottom of the bottle. If these are present dissolve the crystals before use by warming the solution to approximately 30°C – 40°C and mixing by inversion until completely dissolved. To obtain 1X Wash Buffer, dilute the required amount of 10X Wash Buffer Stock Solution **1 in 10** in deionized water. **The 1X Wash Buffer is stable at RT for one week.**
- **Conjugate dilution.** Prepare a **Conjugate pre-dilution by adding 2 µl of Conjugate stock to 1000 µl CDB**. This pre-dilution has to be **further diluted** to obtain the Conjugate working solution (volumes depending on number of microwell strips used, see Table 2

below).
The **Conjugate is provided in a viscous storage buffer containing 50% glycerol**. Thus, **pipette the stock solution carefully** under visual control and make sure that no additional solution is attached to the outside of the pipette tip. Make sure that the 2 µl Conjugate stock is transferred quantitatively to the CDB by pipetting up and down several times and then mix the pre-dilution carefully but thoroughly before preparing the Conjugate working dilution. Always prepare a fresh Conjugate pre-dilution and Conjugate working dilution before performing the test and discard residual pre-dilution and working dilution afterwards. **Afterwards place the Conjugate stock immediately back to -20°C.**

- **HRP-Streptavidin dilution.** Prepare an **HRP-Streptavidin pre-dilution by adding 1 µl of HRP-Streptavidin stock to 100 µl CDB**. Mix carefully but thoroughly. This pre-dilution has to be **further diluted** to obtain the HRP-Streptavidin working solution (volumes depending on number of microwell strips used, see Table 2 below). **Place the HRP-Streptavidin stock back to -20°C immediately after use.**
- **Control samples.** The Control samples are supplied ready-to-use and must not be diluted.
- **Serum samples dilution**. Dilute the serum samples in SDB (50 µl SDB + 1 µl serum).
- **TMB Substrate, Stop Solution.** Both solutions are provided in the kit ready to use (required volume depending on the number of microwell strips used, see Table 2 below).

# strips	Wash Buffer		Conjugate working dilution		HRP-Streptavidin working dilution		Substrate (ml)	Stop Solution (ml)
	10X Wash Buffer (ml)	Dest. water (ml)	Conjugate pre-dilution (µl)	CDB (µl)	HRP-Streptavidin pre-dilution (µl)	CDB (µl)		
1	5	45	20	480	5	495	1	1
2	10	90	20	480	10	990	2	2
3	15	135	30	720	15	1485	3	3
4	20	180	40	960	20	1980	4	4
5	25	225	50	1200	25	2475	5	5
6	30	270	60	1440	30	2970	6	6
7	35	315	70	1680	35	3465	7	7
8	40	360	80	1920	40	3960	8	8
9	45	405	90	2160	45	4455	9	9
10	50	450	100	2400	50	4950	10	10
11	55	495	110	2640	55	5445	11	11
12	60	540	120	2880	60	5940	12	12

Table 2: Preparation of reagents for different numbers of microwell strips used for testing.

ELISA procedure

1. ***Plate and reagents preparation.*** Prepare required number of microwell strips, 1X Wash Buffer, Conjugate dilution and serum samples dilutions as stated above in section “Preparation of reagents and specimen”.
2. ***Initial strips washing.*** Wash strips three times with 300 µl 1X Wash Buffer per well. Soak strips for 30 sec in between washes. Remove residual Wash Buffer by patting upside-down on a paper towel then, without letting the wells dry out, immediately proceed with step 3.
3. ***Adding Conjugate, Control samples and serum samples.*** Pipette 25 µl of Conjugate working solution in each well, then add 25 µl of Control samples (2 wells ready-to-use Negative Control, 1 well ready-to-use Positive Control) and diluted serum samples into the respective wells. Mix carefully by pipetting or by gently tapping the plate with the fingertips. Make sure that the bottom of the wells is completely covered and seal strips with adhesive foil.
4. ***Incubation.*** Incubate the strips overnight (24 h) at 4°C in a sealed, moist environment (wet chamber).
5. ***Preparation of HRP-Streptavidin working solution.*** Prepare HRP-Streptavidin working solution as stated above in section “Preparation of reagents and specimen”.
6. ***Strips washing.*** Wash strips 5 times with 400 µl 1X Wash Buffer per well, allowing the wells to soak for 30 sec with each addition of 1X Wash Buffer, before aspiration/removal of the buffer. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 7.
7. ***Adding HRP-Streptavidin working dilution.*** Add 50 µl HRP-Streptavidin working dilution per well. Make sure that the bottom of the wells is completely covered and seal strips with adhesive foil.
8. ***Incubation.*** Incubate the strips for 1h at 2°C - 8°C in a sealed, moist environment (wet chamber).
9. ***Strips washing.*** Wash strips 5 times with 400 µl 1X Wash Buffer per well. Soak strips for 30 sec in between washes. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 10.
10. ***Substrate incubation.*** Add 100 µl Substrate to each well; incubate for 10 min at room temperature (20°C – 25°C) in the dark. Wells generating a positive result will turn blue.
11. ***Stopping.*** Add 100 µl of Stop Solution to each well. The blue color in wells generating a positive result will turn yellow.
12. ***Measurement.*** Measure optical density at 450 nm and 620 nm using a microplate reader within 30 min after stopping the assay. Calculate the difference OD450 – OD620.

Evaluation of results

Test results can be interpreted as valid if the following criteria are met:

OD450-OD620 of Positive Control > 90% of the value shown on the batch-specific Certificate of Analysis.
OD450-OD620 of Negative Control < 0.1

Use the average absolute OD450-OD620 absorbance value for the Negative Control sample ($OD_{neg, av}$) to calculate the assay cut-off OD_{CO} according to the following formula:

$OD_{neg, av} + 0.150 = OD_{CO}$

The Index Values (IV) for the tested serum samples can then be calculated according to:

IV_{Sample} = OD450-OD620(sample)/OD_{CO}

Evaluate the obtained results according to Table 3.

Index (I)	Result LASV IgG	Interpretation
$I_{Sample} \geq 1.1$	positive	Indicative of past or current infection with LASV, assess positive result by additional testing and/or clinical findings.
$0.9 < I_{Sample} < 1.1$	equivocal	Ambiguous result, repeat test for this serum and (if available) a follow-up sample of the patient taken later and/or analyze sample by additional testing.
$I_{Sample} \leq 0.9$	negative	No anti-LASV IgG antibodies were detected. This finding does not exclude an acute infection with LASV, because IgG antibodies are often not detectable in the first two weeks post onset of illness. If available, a follow-up sample of the patient taken several weeks later should be tested.

Table 3: Result classification for index values and interpretation of results.

Kit shelf life and kit component storage

- Under correct storage conditions (see below), stability of the kit is guaranteed until the expiration date label on the box and/or components. Do not use the kit after the expiration date.
- Upon receipt of the kit**, immediately store the Conjugate (recombinant biotinylated LASV antigen) and the HRP-Streptavidin stock solution at -20°C**. For Conjugate dilution remove the necessary amount of Conjugate from the vial and immediately place the residual Conjugate back in the freezer. As the Conjugate storage buffer contains glycerol, it is not necessary to thaw the Conjugate solution before pipetting. For preparation of the HRP-Streptavidin working solution remove the necessary amount from the stock vial and immediately place the residual stock solution back in the freezer.
- All other kit components have to be stored refrigerated (2°C - 8°C).

General safety information

- The kit is intended for research use only (RUO).
- Tests must be performed by qualified laboratory personnel.
- Wear appropriate protective clothing (lab coat, gloves, safety glasses) when handling kit components and patient sera.
- As they are blood products, Positive and Negative Control samples as well as patient samples should be treated as potentially infectious. It is recommended to handle and dispose of samples and all material having been in contact with the samples (i.e. pipette tips, tubes, microwell strips) under appropriate safety conditions. With the LASV being a virus of biological safety level 4, inactivation of patient sera prior to testing is advised. Inactivation using Triton X-100 is recommended, as described above. **Do not use heat inactivation.**
- The Stop Solution contains sulfuric acid, which is corrosive. Contact with skin or eyes must be avoided. In case of exposure, flush with large amounts of water.

Reference List

[1] Raabe V, Koehler J. (2017). Laboratory diagnosis of Lassa fever. *J Clin Microbiol* 55:1629 –163.

[2] Gabriel M, Adomeh DI, Ehimuan J, Oyakhilome J, Omomoh EO, Ighodalo Y, Olokor T, Bonney K, Pahlmann M, Emmerich P, Lelke M, Brunotte L, Ölschläger S, Thomé-Bolduan C, Becker-Ziaja B, Busch C, Odia I, Ogbaini-Emovon E, Okokhere PO, Okogbenin SA, Akpede GO, Schmitz H, Asogun DA, Günther S (2018). Development and evaluation of antibody-capture immunoassays for detection of Lassa virus nucleoprotein-specific immunoglobulin M and G. *PLoS Negl Trop Dis.* 29;12(3):e0006361

[3] Schmitz H, Gabriel M, Emmerich P (2011) Specific detection of antibodies to different flaviviruses using a new immune complex ELISA. *Med Microbiol Immunol*. 200(4):233-9

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